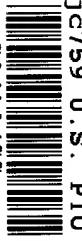


**NONPROVISIONAL PATENT  
APPLICATION TRANSMITTAL RULE §1.53(b)  
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

jc658 U.S. PTO  
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Docket No. 32232-159912  
Date: April 14, 2000



Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

Transmitted herewith for filing under 37 C.F.R. §1.53(b) is a nonprovisional patent application:

For (Title): PROCESS FOR THE SEPARATION OF ENANTIOMERS AND ENANTIOPURE REAGENT

By (Inventors): Thierry DELPLANCHE (Ottignies-Louvain-La-Neuve, Belgium) and Roland CALLENS (Grimbergen, Belgium)

- 34 pages of Specification/Claims 1-16/Abstract are attached.
- Formal drawings (Fig. \_\_\_\_; \_\_\_\_ sheets) are attached.
- A Declaration and Power of Attorney is filed herewith.
- An assignment of the invention to SOLVAY (Société Anonyme), is filed herewith, along with form PTO-1595 and a check for \$40.00.
- An Information Disclosure Statement is filed herewith, along with form PTO-1449, and 1 reference.
- A Statement to establish small entity status under 37 C.F.R. §§1.9 and 1.27 is filed herewith.
- A Preliminary Amendment is filed herewith.
- Please amend the specification by inserting before the first line the sentence --This nonprovisional application claims the benefit of U.S. Provisional Application No. \_\_\_\_\_, filed \_\_\_\_\_.
- Priority of foreign application No. 09900280 filed April 21, 1999 in BELGIUM is claimed (35 U.S.C. §119).
- A certified copy of the above corresponding foreign application is filed herewith.

The filing fee is calculated below and includes claim status after entry of any Preliminary Amendment noted above:

FOR:	NO. FILED	NO. EXTRA
BASIC FEE		
TOTAL CLAIMS	16 - 20	= 0
INDEP CLAIMS	3 - 3	= 0
<input type="checkbox"/> MULTIPLE DEPENDENT CLAIMS		

SMALL ENTITY		LARGE ENTITY	
RATE	FEES	RATE	FEES
	\$ 345		\$ 690
x 9 =	\$	x 18	\$
x 39 =	\$	x 78	\$
+130 =	\$	+260	\$
TOTAL	\$	TOTAL	\$ 690

- A check in the amount of \$730.00 (\$690.00 filing fee and \$40.00 Recordation of Assignment Fee) is attached hereto. Except as otherwise noted herein, the Commissioner is hereby authorized to charge any other fees that may be required to complete this filing, or to credit any overpayment, to Deposit Account No. 22-0261.
- This application is entitled to small entity status. DO NOT charge large entity fees to our Deposit Account.

Respectfully submitted,

Marina V. Schneller  
Registration No. 26,032

Case S 99/08 - Process for the separation of  
enantiomers and enantiopure reagent

SPECIFICATION FILED IN SUPPORT OF A PATENT APPLICATION  
ON BEHALF OF SOLVAY (Société Anonyme)

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Process for the separation of enantiomers and  
enantiopure reagent

The invention relates to a process for the  
5 separation of enantiomers and to reagents based on an  
enantiopure amino acid which can be used in the  
separation of enantiomers.

The separation of enantiomers is a matter of  
great importance in the pharmaceutical, chemical and  
10 biotechnology industries. This is because the two  
enantiomers of a chemical substance with an identical  
composition can have radically different biological  
activities. It is thus desirable to have available  
separation reagents and techniques which make it  
15 possible to separate the enantiomers and to analyse the  
enantiomeric purity of pharmaceutical, chemical and  
biotechnology products.

An article by Marfey, P., (Carlsberg Res.  
Comm., 49, 1984, 591-596) describes a process for the  
20 separation of enantiomers by RP-HPLC. According to this  
known process, 1-fluoro-2,4-dinitrophenyl-5-L-alan-  
inamide is used as reagent for the derivatization of  
amino acids. Other similar processes are also known.  
However, the process and reagent which are described by  
25 Marfey and the other processes and reagents exhibit  
numerous disadvantages.

The derivative obtained in the reaction of the  
amino acid with the reagent has to be isolated by  
successive neutralization, drying, redissolution and  
30 filtration operations. These operations take a great  
deal of time and are therefore not very advantageous in  
an industrial application. Furthermore, there is a  
risk, in cases of analytical applications, of errors in  
the analytical results caused by differing solubility  
35 of the diastereomeric derivatives in the redissolution  
solvent. When quantitative analyses are carried out  
using UV spectrometry, difficulties due to differences  
in absorption coefficient of the diastereomeric  
derivatives are encountered in the known process.

Finally, the high cost of the reagent renders it desirable to find alternatives.

The invention is targetted at overcoming these problems.

5       The invention consequently relates to a process for the separation of enantiomers comprising at least one free functional group, in which

- 10      (a) a mixture comprising the enantiomers is reacted in basic medium with a reagent based on an enantiopure amino acid, in which reagent at least one amino group of the amino acid carries an activating group, in order to form an active precursor of an isocyanate group, and in which reagent at least one carboxyl group of the amino acid is substituted, and
- 15      (b) the mixture of diastereomers obtained is subjected to a separation operation.

It has been found, surprisingly, that the process according to the invention makes it possible to obtain good results with regard to the separation of enantiomers comprising at least one free functional group, in particular in quantitative analytical applications. The process according to the invention makes possible rapid derivatization and rapid separation of enantiomers under flexible and economical conditions.

30      The invention also relates to a reagent based on an enantiopure amino acid in which at least one amino group of the amino acid carries an activating group in order to form an active precursor of an isocyanate group and in which at least one carboxyl group of the amino acid is substituted.

35      The term "amino acid" is understood to denote, for the purposes of the present invention, any compound comprising at least one NH<sub>2</sub> group and at least one carboxyl group. The amino acids used in the present invention are chiral amino acids comprising at least one asymmetric carbon. Use may be made of any chiral

amino acid well known in itself of natural or synthetic origin.

Examples of reagents according to the invention are based, for example, on the following natural amino acids:

alanine, valine, norvaline, leucine, norleucine, isoleucine, serine, isoserine, homoserine, threonine, allothreonine, methionine, ethionine, glutamic acid, aspartic acid, asparagine, cysteine, cystine, phenylalanine, tyrosine, tryptophan, lysine, arginine, histidine, ornithine, glutamine and citrulline.

Unnatural enantiomers can also be used.

Examples of amino acids of synthetic origin which can be used as basis for the reagent according to the invention comprise, for example, the following amino acids: (1-naphthyl)alanine, (2-naphthyl)alanine, homophenylalanine, (4-chlorophenyl)alanine, (4-fluorophenyl)alanine, (3-pyridyl)alanine, phenylglycine, diaminopimelic acid (2,6-diaminoheptane-1,7-dioic acid), 2-aminobutyric acid, 2-aminotetralin-2-carboxylic acid, erythro- $\beta$ -methylphenylalanine, threo- $\beta$ -methylphenylalanine, (2-methoxyphenyl)alanine, 1-amino-5-hydroxyindan-2-carboxylic acid, 2-aminoheptane-1,7-dioic acid, (2,6-dimethyl-4-hydroxyphenyl)-alanine, erythro- $\beta$ -methyltyrosine or threo- $\beta$ -methyltyrosine.

The term "enantiopure amino acid" is understood to denote a chiral amino acid composed essentially of one enantiomer. The enantiomeric excess (ee) is defined: ee (%) =  $100(x_1-x_2)/(x_1+x_2)$  with  $x_1 > x_2$ ;  $x_1$  and  $x_2$  represent the content of enantiomer 1 or 2 respectively in the mixture.

Use is generally made of an enantiopure amino acid with an enantiomeric excess of greater than or equal to 99%. Preference is given to an enantiopure amino acid with an enantiomeric excess of greater than or equal to 99.5%. In a particularly preferred way, use is made of an enantiopure amino acid with an enantiomeric excess of greater than or equal to 99.9%.

Any enantiopure amino acid can be used as basis for the reagent according to the invention. The enantiopure amino acid is preferably selected from the abovenamed amino acids of natural or synthetic origin.

5 Amino acids comprising at least one aromatic nucleus, such as, for example, phenylalanine or its derivatives, are particularly well suited as enantiopure amino acid. In a particularly preferred way, the enantiopure amino acid is selected from phenylalanine, (1-naphthyl)-

10 alanine, (2-naphthyl)alanine or  $\alpha$ - or  $\beta$ -tryptophan ((2-indolyl)alanine or (3-indolyl)alanine), which are optionally substituted.

In the reagent according to the invention, at least one amino group of the enantiopure amino acid carries an activating group in order to form an active precursor of an isocyanate group.

The term "active precursor of an isocyanate group" is understood to denote any precursor which, when it is employed in a solvent which can be used in the process according to the invention with 1 equivalent of phenylalanine in the presence of 1 equivalent of base, reacts at a temperature of less than or equal to 35°C essentially completely in a period of time of less than or equal to 30 min to form the corresponding urea. The reactive precursor preferably releases the isocyanate group at a temperature of less than or equal to 30°C in a period of time of less than or equal to 15 min. In a very particular preferred way, the reactive precursor releases the isocyanate group at room temperature in a period of time of less than or equal to 10 min. Test conditions which can be used to determine the active precursor are described, for example, in Example 3 below.

35 The activating group is generally composed of a carbonyl derivative bonded to an electronegative substituent. Use may be made, for example, as activating group, of an aryloxycarbonyl, heteroaryloxycarbonyl, 1,3-imidazolyl-N-carbonyl or

1,2,4-triazolyl-N-carbonyl group. The aryloxycarbonyl groups which are well suited include those which carry at least one - I, - M substituent on an aromatic nucleus. An - I, - M substituent is a group which has a  
5 negative inductive effect and negative resonance effect as defined in J. March, Advanced Organic Chemistry, 4th Ed., 1992, p. 17-19, 273-275. The - I, - M substituents include, for example,  $-\text{NO}_2$ ,  $-\text{SO}_2\text{R}$ ,  $-\text{SO}_2\text{OR}$ ,  $-\text{NR}_3^+$  and  $\text{SR}_2^+$ . The substituents are preferably found at at least one  
10 of the 2, 4 or 6 positions of the aromatic nucleus or at positions analogous to the 2 or 4 positions in condensed aromatic systems. It is preferable to use an aryloxycarbonyl activating group which carries at least one nitro substituent on the aromatic nucleus. The  
15 (4-nitrophenoxy)carbonyl group is particularly preferred.

In an alternative form, the electronegative substituent comprises an - I substituent (negative inductive effect) independently of the resonance effect  
20 (M) as they have been defined above. In this alternative form, the activating group is preferably an aryloxycarbonyl group carrying at least one - I substituent. The - I substituent is preferably found at the positions which were taught above for the - I, - M substituents. Examples of - I substituents which can be  
25 used in the reagent according to the invention are, for example, halogens. Chlorine and fluorine are well suited. Fluorine is preferred.

In an alternative form of the invention,  
30 everything else being equal, the reagent is based on an enantiopure amino acid in which at least one amino group of the amino acid carries an activating group in order to form an active precursor of an isothiocyanate group. Use may be made, for example, as activating  
35 group, of a thiocarbonyl group bonded to an electronegative group as described above. A (4-nitrophenyloxy)thiocarbonyl or (4-fluorophenyloxy)thiocarbonyl group is preferred.

It has been found that, in this alternative form of the invention, the reaction of the reagent with the organic compound comprising a free functional group generally gives rise to the formation of derivatives 5 comprising a thiocarbonyl group which can give a separation of enantiomers which is further improved with respect to the oxygen-comprising derivatives. The presence of sulphur in the diastereomeric derivatives further facilitates the detection of the said 10 derivatives, in particular by UV spectrometry.

In the reagent according to the invention, at least one carboxyl group of the amino acid is substituted.

The substituent by which the carboxyl group of 15 the amino acid is substituted generally does not comprise a free functional group capable of reacting with the active precursor. The substituents which can be used include, for example, linear or branched alkyl groups comprising from 1 to 4 carbon atoms, such as the 20 methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl or t-butyl group, ethers or aryl groups, the alkyl, ethyl or aryl groups optionally being functionalized by, for example, halogens, carboxylic or sulphonic esters, sulphate esters, phosphonic esters or phosphate 25 esters. Hydrophilic substituents which ensure good solubility of the reagent in mixtures of water with organic solvents are well suited.

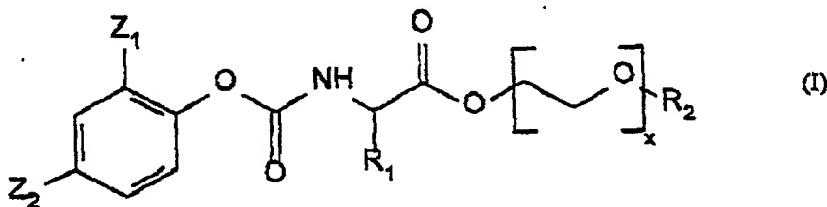
The hydrophilic substituent generally ensures 30 that a solution of the reagent in a 1:1 by volume dioxane/water mixture is homogeneous at 20°C when the concentration of the reagent is at least  $0.5 \times 10^{-3}$  mol/l. The concentration is often at least  $1 \times 10^{-3}$  mol/l. The concentration is preferably at least  $0.5 \times 10^{-2}$  mol/l. Good hydrophilic substituents ensure 35 that the solution is homogeneous at 20°C when the concentration of the reagent is at least  $1 \times 10^{-2}$  mol/l.

It is preferable to employ a substituent which comprises at least one ether bond. Examples of substituents comprising at least one ether bond are,

for example, alkyl or aryl ethers of mono-, oligo- or polyalkylene glycols, such as, for example, mono-, oligo- or polyethylene glycol or mono-, oligo- or polypropylene glycol. The 2-methoxyethyl substituent is  
5 particularly preferred.

Particularly preferred alternative forms of the reagent according to the invention comprising a hydrophilic substituent correspond to the general formula (I)

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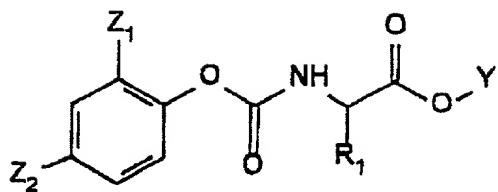


in which  $Z_1$  and/or  $Z_2 = NO_2$ ,  $R_1 =$  phenyl,  $\alpha$ - or  
15  $\beta$ -indolyl, 1-naphthyl or 2-naphthyl,  $R_2 = Me, Et, C_3-C_6$   
alkyl or  $C_3-C_6$  cycloalkyl, and  $x$  represents an integer  
from 1 to 5.

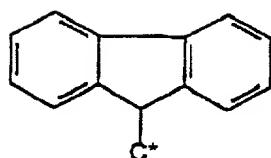
In an alternative form, the substituent by which the carboxyl group or the amino acid is substituted comprises at least one chromophore. The term "chromophore" is understood to denote a functional group which absorbs electromagnetic radiation. The absorption maximum of the chromophores is generally from 170 to 2500 nm. The absorption maximum of the chromophores is preferably from 200 to 1000 nm.  
20 Examples of chromophores which can be used are aromatic systems optionally substituted in the 2 or 4 position by an - I, - M substituent as described above. The 4-nitrobenzyl, (2-anthraquinonyl)methyl and (9-(9H-fluorenylmethyl)) groups are particularly preferred  
25 among the substituents comprising at least one chromophore.  
30

Preferred alternative forms of the reagent according to the invention comprising at least one chromophore correspond to the general formula (II)

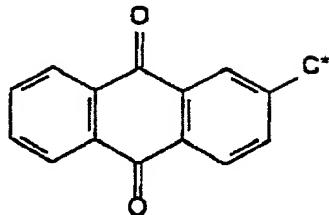
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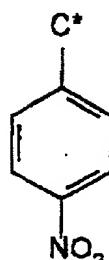
(II)



(III)



(IV)

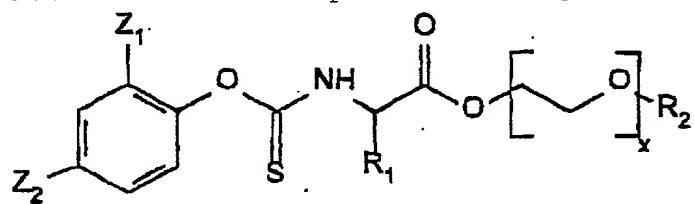


(V)

5 in which  $Z_1$  and/or  $Z_2 = NO_2$ ,  $R_1 =$  phenyl,  $\alpha$ - or  $\beta$ -indolyl, 1-naphthyl or 2-naphthyl and  $Y$  corresponds to any one of the formulae (III to V), the carbon by which  $Y$  is bonded to the oxygen of the carboxyl group of the enantiopure amino acid being marked by  $*$ .

10 Use may be made of a hydrophilic substituent comprising at least one chromophore.

Particularly preferred alternative forms of the reagent according to the invention comprising an active precursor of an isothiocyanate group and a hydrophilic substituent correspond to the general formula (VI)

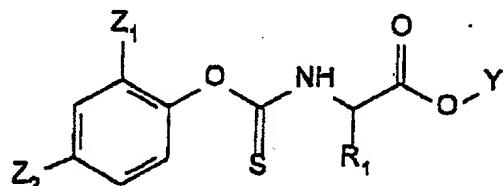


(VI)

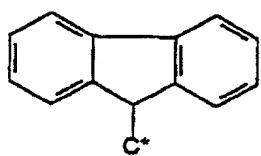
in which  $Z_1$  and/or  $Z_2 = NO_2$  or  $F$ ,  $R_1 =$  phenyl,  $\alpha$ - or  $\beta$ -indolyl, 1-naphthyl or 2-naphthyl,  $R_2 = Me$ ,  $Et$ ,  $C_3-C_6$  alkyl or  $C_3-C_6$  cycloalkyl and  $x$  represents an integer from 1 to 5.

Preferred alternative forms of the reagent according to the invention comprising an active precursor of an isothiocyanate group and at least one chromophore correspond to the general formula (VII)

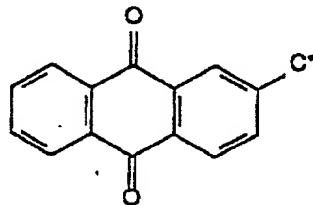
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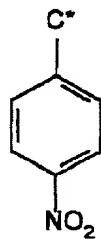
(VII)



(III)



(IV)



(V)

15 in which  $Z_1$  and/or  $Z_2 = NO_2$ ,  $R_1 =$  phenyl,  $\alpha$ - or  $\beta$ -indolyl, 1-naphthyl or 2-naphthyl and  $Y$  corresponds to any one of the formulae (III to V), the carbon by which  $Y$  is bonded to the oxygen of the carboxyl group of the enantiopure amino acid being marked by \*.

20 When the enantiopure amino acid comprises more than one carboxyl group, it is preferable to protect all the carboxyl groups. In a particularly preferred

way, all the carboxyl groups are substituted by a substituent as described above.

When free functional groups are present in the enantiopure amino acid, it is preferable to protect the  
5 said groups by techniques known in themselves.

The reagent according to the invention can be obtained from the respective enantiopure amino acid. Known methods can be used to carry out the esterification of at least one carboxyl group of the  
10 amino acid. It is possible, for example, to employ the enantiopure amino acid and the alcohol corresponding to the substituent to be introduced in an organic solvent, such as, for example, toluene or benzene, in the presence of p-toluenesulphonic acid, preferably under  
15 azeotropic esterification conditions. Amino acid ester ammonium tosylate derivatives which are well suited to the introduction of an activating group in order to form an active precursor of an isocyanate group are obtained by this synthetic route.

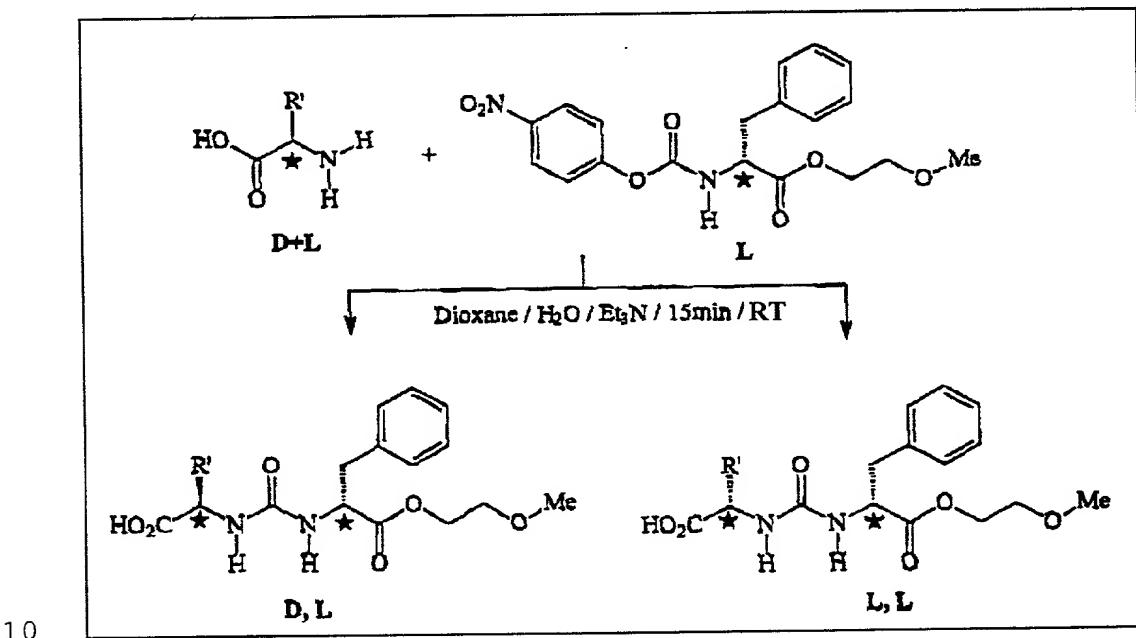
20 Mention is made, as example of the introduction of an activating group, of the reaction of an aryloxycarbonyl chloride with the -NH<sub>2</sub> group, optionally converted to the ammonium derivative, of an amino acid or of an amino acid ester in basic or neutral medium in  
25 a polar organic solvent. Tertiary amine bases, such as, for example, triethylamine or pyridine, are suitable in particular as base. When the operation is carried out in neutral medium, it is preferable to employ sodium hydrogencarbonate. Thus, good results are obtained when  
30 an amino acid ester ammonium tosylate is reacted with p-nitrophenyloxycarbonyl chloride in the presence of sodium hydrogencarbonate in a polar organic solvent, such as acetonitrile.

In the process according to the invention, the  
35 reagent according to the invention is reacted in basic medium with a mixture comprising at least enantiomers comprising at least one free functional group.

The reaction of a reagent according to the invention, obtained from the 2-methoxyethyl ester of

L-phenylalanine and from 4-nitrophenyl chloroformate, with an amino acid is illustrated in a non-limiting way in Scheme 1 below. The products of this reaction are ureas comprising two amino acids, in which at least one carboxyl functional group is substituted with a substituent.

Scheme 1



Generally, the free functional group is an optionally monoalkylated amino group, a hydroxyl group or a thiol group. The free functional group can also be composed of an anion, such as, for example, a carbanion or an enolate. The enantiomers comprising at least one free functional group which can be separated by the process according to the invention are generally amino acids, primary or secondary amines, peptides, alcohols, hydroxy acids or thiols. The process according to the invention gives good results in separating the enantiomers of amino acids, such as, for example, the amino acids of natural or synthetic origin mentioned above.

The process according to the invention also gives good results in separating a mixture of

enantiomers of imino acids. The term "imino acid" is understood to denote any compound comprising at least one NHR group, in which R represents an organic radical, such as, for example, an alkyl or aryl radical, and at least one carboxyl group. Such imino acids are, for example, those belonging to the group composed of proline, pipecolic acid (piperidine-2-carboxylic acid), morpholine-3-carboxylic acid, piperazine-2-carboxylic acid, 1-thia-4-azacyclohexane-3-carboxylic acid,  $\alpha$ -methylproline, cis-4-hydroxyproline, baikaine (1,2,3,5-tetrahydropyridine-2-carboxylic acid), cis-4-hydroxypipeolic acid, trans-5-hydroxypipeolic acid, 1,2,3,4-tetrahydronorharman-1-carboxylic acid, 1,2,3,4-tetrahydro-6-hydroxyisoquinoline-3-carboxylic acid, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, 1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid and N-methylvaline.

The process according to the invention is carried out in a solvent system in which the mixture of enantiomers and the reagent possess sufficient solubility and the free functional group possesses sufficient nucleophilicity to react with an isocyanate. Systems comprising at least one polar organic solvent and optionally water are suitable, for example, as solvent system. Polar organic solvents which can be used are, for example, aliphatic or alicyclic ethers, such as diethyl ether, tetrahydrofuran or 1,4-dioxane, as well as aliphatic esters, such as, for example, ethyl acetate, aliphatic secondary amides, such as, for example, dimethylformamide and dimethylacetamide or, for example, N-methylpyrrolidone, or acetonitrile.

Good results for organic compounds comprising a free functional group, such as an optionally monoalkylated amino group, an arylic hydroxyl group or a thiol group, are obtained in a solvent system comprising a polar organic solvent and water. Dioxane is preferred as polar organic solvent. The ratio by weight of the polar organic solvent to the water in the solvent system is generally less than or equal to 99:1.

The ratio is most often less than or equal to 75:25. The ratio is generally greater than or equal to 1:99. The ratio is most often greater than or equal to 25:75.

The invention also relates to a solution of the reagent according to the invention in a polar organic solvent, such as, for example, the polar organic solvents described above. The concentration of the reagent in the solution is generally at least  $1 \times 10^{-3}$  mol/l. The concentration is preferably at least  $1 \times 10^{-2}$  mol/l. The concentration of the reagent in the solution is generally at most  $1 \times 10^{-1}$  mol/l. The concentration is preferably at most  $6 \times 10^{-2}$  mol/l. In the solution according to the invention, it is preferable to use a polar organic solvent of analytical purity. If desirable, the solution according to the invention can comprise additives, such as, for example, stabilizers.

The invention also relates to the use of the solution according to the invention in an automatic device for the derivatization and separation of enantiomers of organic compounds comprising at least one free functional group. The amount of the reagent to be employed depends on the number of free functional groups in the organic compound. At least 1 molar equivalent of reagent is employed per free functional group. Generally, at most 10 molar equivalents of reagent are employed per free functional group. Most often, at least 5 molar equivalents of reagent are employed per free functional group. Preferably, at most 3 molar equivalents of reagent are employed per free functional group. In a particularly preferred way, from 1.1 to 2.5 molar equivalents of reagent are employed per free functional group.

The basic nature of the reaction mixture is generated by known methods. The operation is preferably carried out in the presence of at least one base. Tertiary amine bases, such as, for example, triethylamine or diisopropylethylamine, which comprise one basic functionality respectively, or N,N,N',N'-

tetramethylethylenediamine, which comprises 2 basic functionalities, are suitable in particular as base.

The amount of base to be employed depends on the amount of the reagent and on the number of basic 5 functionalities in the base. The molar ratio of the reagent to the basic functionalities is generally at least 1. The ratio is generally at most 2. A ratio of 1 gives good results.

In the process according to the invention, the 10 period of time during which the reagent is reacted with the mixture comprising the enantiomers is generally less than or equal to 30 min. Most often, the period of time is less than or equal to 20 min. Preferably, the period of time is less than or equal to 15 min. Good 15 results are obtained with a period of time of greater than or equal to 15 seconds. In practice, a period of time of greater than or equal to 1 min is most often applied. A period of time of 5 to 15 min is highly suitable.

20 The temperature at which the reagent is reacted with the mixture comprising at least the enantiomers of an organic compound is generally less than or equal to 35°C. The temperature is most often less than or equal to 30°C. The temperature is generally greater than or 25 equal to -20°C. The temperature is most often greater than or equal to 0°C. In a particularly preferred way, the temperature is room temperature, that is to say generally from 15 to 30°C, preferably 20 to 25°C.

In the process according to the invention, the 30 mixture of diastereomers obtained is subjected to a separation operation. The separation operations which can be used for the separation of a mixture of diastereomers are described, for example, in E. Eliel, Stereochemistry of Organic Compounds, 1994, p. 344-381. 35 Mention may be made, as examples, of distillation, crystallization and gas or liquid chromatography operations. Among these operations, liquid chromatography operations, such as, for example, HPLC chromatography, are preferred. In a particularly

preferred way, the separation operation is RP-HPLC (reverse phase) chromatography. Information regarding HPLC chromatography is found, for example, in Rompp Chemie-Lexikon, 9th Ed., p. 1860-1861. Use may also be  
5 made of thin layer chromatography.

Eluents which can be used in a chromatography operation are known. In the case where the process according to the invention comprises RP-HPLC chromatography as separation operation, good results  
10 have been obtained with an eluent comprising acetonitrile or methanol.

In an alternative form of the process according to the invention, which is preferred, the mixture of diastereomers obtained is subjected to the separation  
15 operation without prior purification. In known methods for the separation of enantiomers, a crude mixture of diastereomers is isolated, which mixture has to be subjected to purification prior to the separation of the diastereomers. The process and the reagent  
20 according to the invention make it possible not to isolate the crude mixture of diastereomers and to carry out the separation operation without prior purification.

The process and the reagent according to the  
25 invention can be used for the preparative or analytical separation of enantiomers. The process and the reagents are well suited to the analytical separation of enantiomers. In an alternative form, the process and the reagent are used to determine the enantiomeric excess of an amino acid or of a primary or secondary amine. In another alternative form, the process and the reagent are used to determine the enantiomeric excess  
30 of a peptide.

The invention also relates to a process for the  
35 production of an enantiopure compound comprising at least one free functional group in which:

- (a) a mixture comprising the enantiomers of the compound comprising at least one free functional

group is subjected to the separation process according to the invention

- (b) a cleavage operation is carried out on a pure diastereomer obtained by separation of the mixture  
5 of diastereomers  
(c) the enantiopure compound is recovered.

Use may be made, as cleavage operation, of, for example, an operation of hydrazinolysis in a solvent, such as, for example, an alcohol.

10 When the process and the reagent according to the invention are used for the analytical separation of enantiomers, use is made of a detection technique known in itself for the determination of the content of enantiomers in the mixture. Optical techniques, such  
15 as, for example, UV spectrometry, visible spectrometry or fluorimetry are highly suitable as detection technique.

The examples below are intended to illustrate the invention without, however, limiting it.

20 Example 1 (Synthesis of the reagent according to the invention)

Stage A

25 1 equivalent of enantiopure amino acid, 1.5 equivalents of para-toluenesulphonic acid monohydrate, 50 equivalents of toluene PA and 5 equivalents of methoxyethanol were introduced into a single-necked round-bottomed flask. The mixture was heated to reflux and the water was removed by means of a Dean and Stark apparatus. After refluxing for  
30 4 hours, the reaction mixture was cooled to room temperature and diluted with ethyl ether. The diluted reaction mixture was placed in a refrigerator for 16 hours. The white solid obtained was filtered off, washed with ether and dried under vacuum.

35 Stage B

NaHCO<sub>3</sub> (2.6 equivalents) were weighed into a single-necked round-bottomed flask and acetonitrile was introduced under a stream of nitrogen ( $5 \times 10^{-3}$  mol in 36 ml). The mixture was cooled to 0°C and 4-nitrophenyl

chloroformate (1 equivalent), followed by the ammonium salt of the enantiopure amino acid obtained according to Example 1, Stage A (1 equivalent), was successively introduced. The mixture was vigorously stirred for  
5 1 hour at 0°C and was subsequently brought back to room temperature for 4 hours. At the end of this time, the mixture was transferred into a separating funnel, was diluted with a 1 molar HCl solution and was extracted three times with ether. The combined organic phases  
10 were dried over MgSO<sub>4</sub>, filtered and concentrated under reduced pressure. The crude product was subjected to an appropriate purification treatment.

Example 2

Stages A and B were carried out by employing  
15 L-phenylalanine as enantiopure amino acid. The crude product was recrystallized from isopropanol until the desired purity was obtained. The yield of Stage B was 64%. A crystalline solid was obtained which was stable on storage at room temperature.

20 The reagent thus obtained exhibited the following analytical data:

NMR ( <sup>1</sup> H)	:	(dioxane reference at 3.71 ppm; product dissolved in d <sub>6</sub> -dioxane)
8.40 (2H, d)	:	2H of the (4-nitrophenyloxy) carbonyl
25 7.44 (7H, m)	:	2H of the (4-nitrophenyloxy) carbonyl and 5H of the phenyl
7.31 (1H, d)	:	NH of the carbamate
4.83 (1H, m)	:	CH of the phenylalanine
4.41 (2H, m)	:	CH <sub>2</sub> CO=O
30 3.70 (2H, m)	:	CH <sub>2</sub> OMe
3.47 (3H, s)	:	CH <sub>3</sub> O
3.27 (2H, AB)	:	benzyl 2H

Nuclear magnetic resonance (NMR) was performed,  
in all cases for which an NMR spectrum is shown, with a  
35 Brucker AMX 500 MHz device spectrometer. The chemical shifts are shown in ppm with respect to the resonance of TMS (tetramethylsilane). The following abbreviations are used for the appearance of the resonances:  
m = multiplet, s = singlet, d = doublet, t = triplet

and q = quartet. The number nH indicates the number of protons corresponding to the signal.

Melting point : 71-72°C

Optical rotation :  $[\alpha]: + 42.608$  ( $c = 0.86$  g/  
5 100 ml in dioxane),  $T^\circ = 26^\circ\text{C}$ ,  
 $\lambda = 589$  nm)

Thin layer chromatography (TLC) was performed using Merck® 60F-254 silica gel plates. Retention factor, = 0.45 (diethyl ether/petroleum ether eluent: 10 75/25 mixture by volume).

Example 3-48 (Derivatization and separation of mixtures of enantiomers)

The mixture of enantiomers of an organic compound comprising at least one free functional group according to Examples 3-48 (see Table) (1 equivalent) was weighed accurately in a 10 ml vial and was dissolved in distilled water (concentration  $5 \times 10^{-3}$  mol/l) in the presence of triethylamine. The molar ratio of the triethylamine with respect to the reagent was 1. The reagent obtained according to Example 1 in which the enantiopure amino acid was L-phenylalanine was introduced into a second 10 ml vial. The reagent was dissolved in dioxane of analytical purity (concentration  $3 \times 10^{-2}$  mol/l). The aqueous solution of the amino acid was transferred via a syringe and with vigorous stirring into the solution of the derivatization reagent. After stirring for 15 minutes at room temperature, 500 µl of the reaction mixture was withdrawn and was diluted with 500 µl of dioxane. This solution was analyzed by reverse-phase HPLC by injection of 10 µl of solution onto a Vydac® column.

The following standard conditions were used for the examples of the separation of mixtures of enantiomers by HPLC.

A Vydac® Reverse Phase C18 CAT 201TP54 column was used.

An acetonitrile/H<sub>2</sub>O mixture with an acetonitrile gradient of 1.58%/min in the presence of 0.1% by volume of trifluoroacetic acid was employed for the elution.

Detection was performed by UV spectrometry at 5 205 and 220 nm.

The mixtures of enantiomers which were employed with the reagent obtained according to Example 1 and the separation results obtained are collated in Table 1 below.

10 Table 1

Ex.	Mixture of enantiomers	T <sub>r</sub> L,L <sup>(a)</sup>	T <sub>r</sub> D,L <sup>(b)</sup>	α <sup>(c)</sup>	R <sub>s</sub> <sup>(d)</sup>	Amount of reagent <sup>(e)</sup> (Equivalents)
3	Alanine	15.08	16.40	1.096	6.01	2
4	Valine	18.73	20.94	1.127	10.83	2
5	Norvaline	19.28	21.26	1.11	9.45	2
6	Leucine	21.53	23.50	1.098	8.96	2
7	Norleucine	22.06	23.96	1.091	8.78	2
8	Isoleucine	21.08	23.46	1.120	11.55	2
9	Threonine	14.39	15.41	1.078	4.76	2
10	Allothreonine	14.39	14.85	1.036	2.09	2
11	Methionine	18.97	20.59	1.092	7.74	2
12						
13	Glutamic acid	14.56	15.00	1.033	2.07	2
14	Aspartic acid	14.22	14.66	1.034	2.08	2
15	Cysteine	26.65	27.19	1.021	2.85	5*
16	Cystine	25.59	26.08	1.020	2.76	4*
17	Proline	17.09	17.75	1.042	2.75	2
18	Phenylalanine	22.64	24.08	1.068	6.25	2
19	Tyrosine	28.82	29.24	1.015	1.99	5*
20	Tryptophan	22.79	24.03	1.058	6.54	2
21	Ornithine	24.44	24.92	1.021	2.26	6*
22	Piperidine-2-carboxylic acid (pipecolic acid)	19.86	20.62	1.041	3.51	2

Ex.	Mixture of enantiomers	$T_r L, L^{(a)}$	$T_r D, L^{(b)}$	$\alpha^{(c)}$	$R_s^{(d)}$	Amount of reagent <sup>(e)</sup> (Equivalents)
23	Morpholine-3-carboxylic acid	16.05	15.56	1.034	2.28	2
24	1-Thia-4-aza-cyclohexane-3-carboxylic acid	18.76	19.43	1.038	3.30	2
25	(2-Naphthyl)-alanine	27.43	28.53	1.042	5.19	2
26	Homophenyl-alanine	24.71	26.20	1.064	7.94	2
27	(4-Chlorophenyl)-alanine	25.91	27.02	1.053	5.58	2
28	(4-Fluorophenyl)-alanine	23.65	25.033	1.062	6.53	2
29	(3-Pyridyl)-alanine	14.32	13.57	1.061	3.20	2
30	Phenylglycine	20.98	22.69	1.087	8.24	2
31	2-Methyl-proline	19.29	20.55	1.07	5.76	2
32	cis-4-Hydroxyproline	13.92	14.44	1.041	2.19	2
33	Baikaine	19.05	20.27	1.069	6.03	2
34	cis-4-Hydroxypipercolic acid	15.12	15.91	1.058	3.97	2
35	trans-5-Hydroxypipercolic acid	13.76	14.36	1.049	2.70	2
36	2-Amino-butyric acid	16.84	18.64	1.116	8.31	2
37	1,2,3,4-	23.71	24.75	1.046	4.89	2

Ex.	Mixture of enantiomers	$T_r L, L^{(a)}$	$T_r D, L^{(b)}$	$\alpha^{(c)}$	$R_s^{(d)}$	Amount of reagent <sup>(e)</sup> (Equivalents)
	Tetrahydro-isoquinoline-3-carboxylic acid					
38	1,2,3,4-Tetrahydro-isoquinoline-1-carboxylic acid	23.66 <sup>s</sup>	24.67 <sup>s</sup>	1.045	4.75	2
39	erythro- $\beta$ -Methylphenylalanine	24.03 <sup>s</sup>	25.73 <sup>s</sup>	1.075	8.67	2
40	threo- $\beta$ -Methylphenylalanine	23.79 <sup>s</sup>	25.56 <sup>s</sup>	1.079	7.22	2
41	$\alpha$ -Methoxy-phenylalanine	23.08 <sup>s</sup>	24.31 <sup>s</sup>	1.057	5.64	2
42	1,2,3,4-Tetrahydro-norharman-1-carboxylic acid	26.26 <sup>s</sup>	27.09 <sup>s</sup>	1.031	3.44	5*
43	1,2,3,4-Tetrahydro-6-hydroxy-isoquinoline-3-carboxylic acid	29.14 <sup>s</sup>	29.67 <sup>s</sup>	1.019	2.49	5*
44	2-Amino-heptane-1,7-dioic acid	18.09 <sup>s</sup>	19.21 <sup>s</sup>	1.067	6.41	2
45	erythro- $\beta$ -Methyl-tyrosine	29.84 <sup>s</sup>	30.53 <sup>s</sup>	1.024	3.65	5*
46	threo- $\beta$ -	29.51 <sup>s</sup>	30.07 <sup>s</sup>	1.02	2.97	5*

Ex.	Mixture of enantiomers	$T_r L, L^{(a)}$	$T_r D, L^{(b)}$	$\alpha^{(c)}$	$R_s^{(d)}$	Amount of reagent <sup>(e)</sup> (Equivalents)
	Methyl-tyrosine					
47	N-Methyl-valine	21.47	22.59	1.056	5.62	2
48	2-Hydroxy-, methyl-piperidine	19.79	20.4	1.033	2.73	2

Key for Tables 1 to 6

(a): Retention time corresponding to the derivative of the L enantiomer (L,L)

5 (b): Retention time corresponding to the derivative of the D enantiomer (D,L)

(c): Separation factor;  $\alpha = (T_{r2}-T_{r0})(T_{r1}-T_{r0})$ , where  $T_{r2}$  and  $T_{r1}$  are the retention times of the second and first compounds respectively and  $T_{r0}$  is the 10 retention time of a non-retained compound.

(d): Peak resolution:

$$R_s = 0.25 \left( \frac{\alpha-1}{\alpha} \right) \left( \frac{k'^2}{1+k'^2} \right) \sqrt{N_2}$$

where  $k'^2$  is the capacity factor of the second compound and  $N$  is the number of theoretical plates.

\* Derivatization also of the functional group carried by the side chain.

\$ The attribution of the retention times to the (L,L) and (D,L) isomers respectively is uncertain as the 20 mixture of the enantiomers subjected to the separation was racemic.

Example 49

Stages A and B were carried out by employing L-(2-naphthyl)alanine as enantiopure amino acid. The 25 crude product was recrystallized from isopropanol until the desired purity was obtained. The yield of Stage B was 77%. A crystalline solid was obtained which was stable on storage at room temperature.

The following NMR spectrum of the reagent was obtained:

NMR ( <sup>1</sup> H)	:	(dioxane reference at 3.71 ppm; product dissolved in d <sub>6</sub> -dioxane)
5    8.20 (2H, d)	:	2H of the (4-nitrophenoxy)carbonyl
7.79 (4H, m)	:	4H of the naphthyl
7.45 (3H, d)	:	3H of the naphthyl
7.21 (3H, m)	:	2H of the (4-nitrophenoxy)carbonyl and 1H of the naphthyl
10    4.78 (1H, m)	:	CH of the naphthylalanine
4.27 (2H, m)	:	CH <sub>2</sub> CO=O
3.52 (2H, m)	:	CH <sub>2</sub> OMe
3.28 (3H, s)	:	CH <sub>3</sub> O
3.29 (2H, AB)	:	benzyl 2H
15    Melting point	:	76-77°C

Optical rotation : [α]: 83.5 (c = 1.025 g/100 ml in  
dioxane, T° = 26°C, λ = 589 nm)

Thin layer chromatography (TLC) was performed by using Merck® 60F-254 silica gel plates. Former 20 retention factor = 0.4 (diethyl ether/petroleum ether eluent: 75/25 mixture by volume).

The reaction of the 2-methoxyethyl ester of L-N-(4-nitrophenoxy)carbonyl-(2-naphthyl)alanine with a mixture of arginine enantiomers and the separation 25 operation was carried out under the conditions of Examples 3-48. 2 equivalents of reagent and 2 equivalents of triethylamine per equivalent of arginine were employed.

The separation result obtained is shown in 30 Table 2 below.

Table 2

Ex.	Mixture of enantiomers	T <sub>r</sub> L,L <sup>(a)</sup>	T <sub>r</sub> D,L <sup>(b)</sup>	α <sup>(c)</sup>	R <sub>s</sub> <sup>(d)</sup>	Amount of reagent <sup>(e)</sup> (Equivalents)
49	Arginine	20.168	19.625	1.030	1.73	2

Example 50

The reaction of the 2-methoxyethyl ester of L-N-(4-nitrophenoxy)carbonyl- $\beta$ -tryptophan with a mixture of valine enantiomers and the separation 5 operation were carried out under the conditions of Examples 3-48. 2 equivalents of reagent and 2 equivalents of triethylamine per equivalent of valine were employed.

10 The separation result obtained is shown in Table 3 below.

Table 3

Ex.	Mixture of enantiomers	$T_r$ L,L <sup>(a)</sup>	$T_r$ D,L <sup>(b)</sup>	$\alpha^{(c)}$	$R_s^{(d)}$	Amount of reagent <sup>(e)</sup> (Equivalents)
50	Valine	19.19	20.42	1.069	5.80	2

Example 51: Preparation of the 4-nitrobenzyl ester of N-((4-nitrophenoxy)carbonyl)phenylalanine

15

Stage A:

4 g (1 eq) of Z-(L)-phenylalanine, 2.89 g (1 eq) of 4-nitrobenzyl bromide and 26 ml (25 eq) of dimethylformamide were introduced into a single-necked 20 round-bottomed flask. 1.55 g (2 eq) of dry potassium fluoride were then added under nitrogen. The mixture was stirred at 60°C for 16 hours. The reaction mixture was cooled to room temperature and diluted with 100 ml of ethyl acetate. The organic phase was washed with two 25 times 100 ml of a 5% NaHCO<sub>3</sub> solution and with two times 100 ml of water. The organic phase was dried over MgSO<sub>4</sub>, filtered and concentrated under reduced pressure. The solid obtained was dried in an oven overnight.

Yield: 6.3 g (85%)

30 Stage B:

The solid obtained in Stage 1 was diluted in 25 ml of glacial acetic acid. 6.9 ml (3 eq) of a 33% by weight solution of HBr in acetic acid were carefully added. The mixture was stirred for one and a half hours

at room temperature. The mixture was diluted with 200 ml of ethyl ether and the white precipitate formed was filtered off and washed with three times 200 ml of ethyl ether. The solid was dried in an oven overnight.

5 Yield: 5.53 g (99%)

Stage C:

0.82 g of NaHCO<sub>3</sub> (2.6 eq) was weighed in a single-necked round-bottomed flask and 27 ml of acetonitrile (135 eq) were introduced under a stream of nitrogen. The mixture was cooled to 0°C and 0.81 g (1 eq) of 4-nitrophenyl chloroformate, followed by 1.5 g (1 eq) of the bromine salt of the phenylalanine-4-nitrobenzyl derivative, were successively introduced. The mixture was stirred vigorously for 1 hour at 0°C and was subsequently brought back to room temperature for 11 hours. At the end of this time, the mixture was transferred into a separating funnel, was diluted with 60 ml of a 1M hydrochloric acid solution and was extracted with three times 60 ml of ethyl acetate. The combined organic phases were dried over MgSO<sub>4</sub>, filtered and concentrated under reduced pressure to give a beige solid. The latter was triturated in 50 ml of ether and filtered off.

Yield : 1.44 g (82%)

25	NMR ( <sup>1</sup> H)	: (methanol reference at 3.32 ppm; product dissolved in d <sub>4</sub> -methanol)
	8.32 (2H, d)	: 2H of the (4-nitrophenyloxy)-carbonyl
	8.28 (2H, d)	: 2H of the 4-nitrobenzoyl
30	7.63 (2H, d)	: 2H of the 4-nitrophenyloxycarbonyl
	7.34-7.41 (7H, m)	: 2H of the 4-nitrobenzyl and 5H of the phenyl
	5.37 (2H, s)	: benzyl CH <sub>2</sub> of the 4-nitrobenzyl
	4.65 (1H, m)	: CH of the phenylalanine
35	3.23 (2H, AB)	: benzyl CH <sub>2</sub> of the phenylalanine
	Melting point	: 118.3°C
	Optical rotation	: [α]: + 20.83 (c = 0.96 g/100 ml in dioxane, T° = 26°C, λ = 589 nm)

Thin layer chromatography (TLC) was performed by using Merck® 60F-254 silica gel plates. Retention factor = 0.4 (diethyl ether/petroleum ether eluent: 75/25 mixture by volume).

5    Example 52

The result of the separation of a mixture of valine enantiomers which was carried out with the reagent obtained according to Example 51 according to the procedure of Example 3 is presented in Table 4.

10      Detection was performed by UV spectrometry at 205, 220 and 270 nm.

Table 4

Ex.	Mixture of enantio-mers	T <sub>r</sub> L,L <sup>(a)</sup>	T <sub>r</sub> D,L <sup>(b)</sup>	α <sup>(c)</sup>	R <sub>s</sub> <sup>(d)</sup> (nm)	Equivalents of reagents	R <sub>s</sub> (nm)
52	Valine	26.207	27.246	1.042	3.92 (205 nm)	2	4.77 (220-270)

It was observed that this reagent makes possible detection at 220 nm and 270 nm. A greater separation factor was obtained at these wavelengths than when detection was carried out at 205 nm.

15    Example 53

The 2-methylanthraquinone ester of N-((4-nitrophenoxy)carbonyl)phenylalanine was synthesized according to the procedure of Example 51. The result of the separation of a mixture of valine enantiomers which was carried out with the reagent obtained according to Example 51 according to the procedure of Example 3 is presented in Table 5.

20      The detection was performed by UV spectrometry at 205, 220, 270 and 330 nm.

Table 5

Ex.	Mixture of enantiomers	T <sub>r</sub> L,L <sup>(a)</sup>	T <sub>r</sub> D,L <sup>(b)</sup>	α <sup>(c)</sup>	R <sub>s</sub> <sup>(d)</sup> (nm)	Equivalents of reagents	R <sub>s</sub> (nm)
52	Valine	30.168	30.884	1.025	3.01 (205 nm)	2	3.11 (270 and 330)

It was observed that this reagent makes possible detection at 270 nm and 330 nm. A slightly greater separation factor was obtained at these wavelengths than when detection was performed at 205 nm.

It is apparent that the reagent according to the invention can be easily obtained. The reagent according to the invention exhibits good stability at room temperature.

The process according to the invention makes it possible to separate a greater variety of chiral organic compounds comprising at least one free functional group in a simple and fast way and under uniform separation conditions without having to isolate the reaction product prior to the separation stage.

Example 54

NaHCO<sub>3</sub> (2.6 equivalents) was weighed in a single-necked round-bottomed flask and acetonitrile was introduced under a stream of nitrogen ( $12 \times 10^{-3}$  mol in 83 ml). The mixture was cooled to 0°C and 4-fluorophenyl chlorothioformate (1 equivalent), followed by the enantiopure amino acid ammonium salt obtained according to Example 2, Stage A (1 equivalent), were successively introduced. The mixture was stirred vigorously for 1 hour at 0°C and was subsequently brought back to room temperature for 4 hours. At the end of this time, the mixture was transferred into a separating funnel, was diluted with a 1 molar HCl solution and was extracted three times with ether. The combined organic phases were dried over

MgSO<sub>4</sub>, filtered and concentrated under reduced pressure.

The crude product was subjected to an appropriate purification treatment.

The reagent thus obtained was (S)-N-(4-fluorophenoxy-thiocarbonyl)phenylalanine methoxyethyl ester. It exhibited the following analytical data:

NMR (<sup>1</sup>H) : (dioxane ref. at 3.71 ppm; product dissolved in d<sub>6</sub>-dioxane)

8.88 (2H, d) : 2H of the 4-fluorophenoxythiocarbonyl

10 7.20-7.49 (7H, m) : 2H of the fluorophenoxythiocarbonyl

and 5H of the phenyl

6.99 (1H, d) : NH of the carbamate

5.36 (1H, m) : CH of the phenylalanine

15 4.42 (2H, m) : CH<sub>2</sub>OC=O

3.69 (2H, m) : CH<sub>2</sub>OMe

3.48 (3H, s) : CH<sub>3</sub>O

3.41 (2H, AB) : benzyl 2H

Examples 55-57

20 The derivatization and separation of the mixtures of enantiomers of organic compounds comprising a free functional group of Examples 55-57 (Table) were carried out under the conditions of Examples 3-48 by using, as reagent, the reagent obtained in Example 54.

25 Detection was performed only at 245 nm. The separation results obtained are shown in the table below.

Table 6

Ex.	Mixture of enantiomers	T <sub>r</sub> L,L <sup>(a)</sup>	T <sub>r</sub> D,L <sup>(b)</sup>	$\alpha$ <sup>(c)</sup>	R <sub>s</sub> <sup>(d)</sup>	Amount of reagent (Equivalents)
55	Valine	24.79	22.11	1.129	12.89	2
56	Proline	19.42	21.22	1.099	7.68	2
57	Tyrosine	22.14	23.26	1.054	4.4	5

30 The reagent makes possible very efficient separation of enantiomers. Detection by UV spectrometry can be performed at a single wavelength and it is highly sensitive.

CLAIMS

1. A process for the separation of enantiomers comprising at least one free functional group, in which
- 5 (a) a mixture comprising the enantiomers is reacted in basic medium with a reagent based on an enantiopure amino acid, in which reagent at least one amino group of the amino acid carries an activating group, in order to form an active precursor of an isocyanate group, and in which reagent at least one carboxyl group of the amino acid is substituted, and
- 10 (b) the mixture of diastereomers obtained is subjected to a separation operation.
2. The process according to Claim 1, in which the carboxyl group of the amino acid is substituted with a hydrophilic substituent and/or a substituent comprising at least one chromophore.
- 15 3. The process according to Claim 2, in which the hydrophilic substituent in the reagent is a 2-methoxyethyl group.
- 20 4. The process according to Claim 1, in which the activating group in the reagent is a (4-nitro-phenyloxy)carbonyl group.
- 25 5. The process according to Claim 1, in which the reagent is based on an enantiopure amino acid selected from the group consisting of alanine, valine, norvaline, leucine, norleucine, isoleucine, serine, isoserine, homoserine, threonine, allothreonine, methionine, ethionine, glutamic acid, aspartic acid, asparagine, cysteine, cystine, phenylalanine, tyrosine, tryptophan, lysine, arginine, histidine, ornithine, glutamine, citrulline, (1-naphthyl)alanine, (2-naphthyl)alanine, homophenylalanine, (4-chloro-phenyl)alanine, (4-fluoro-phenyl)alanine, (3-pyridyl)alanine, phenylglycine, dia-
- 30 minopimelic acid (2,6-diaminoheptane-1,7-dioic acid), 2-aminobutyric acid, 2-aminotetralin-2-carboxylic acid, erythro- $\beta$ -methylphenylalanine, threo- $\beta$ -methylphenylalanine, (2-methoxyphenyl)alanine, 1-amino-5-hydroxy-
- 35

indan-2-carboxylic acid, 2-aminoheptane-1,7-dioic acid, (2,6-dimethyl-4-hydroxyphenyl)alanine, erythro- $\beta$ -methyltyrosine and threo- $\beta$ -methyltyrosine.

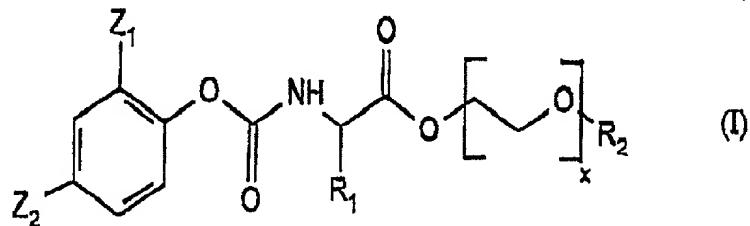
6. The process according to Claim 1, in which the mixture of enantiomers is reacted with the reagent at room temperature for a period of time of less than or equal to 15 minutes and the mixture of diastereomers obtained is subjected to the separation operation without prior purification.

10 7. The process according to Claim 1, in which the separation operation is HPLC chromatography.

8. A reagent based on an enantiopure amino acid in which at least one amino group of the amino acid carries an activating group in order to form an active precursor of an isocyanate or isothiocyanate group and in which at least one carboxyl group of the amino acid is substituted.

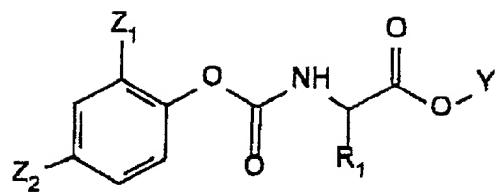
15 9. The reagent according to Claim 8, in which at least one amino group of the enantiopure amino acid carries an activating group in order to form an active precursor of an isocyanate group.

20 10. The reagent according to Claim 9 corresponding to the general formula (I)

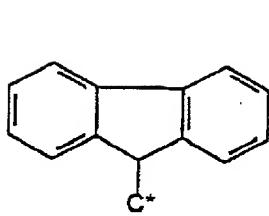


25 in which  $Z_1$  and/or  $Z_2$  =  $\text{NO}_2$ ,  $R_1$  = phenyl,  $\alpha$ - or  $\beta$ -indolyl, 1-naphthyl or 2-naphthyl,  $R_2$  = Me, Et,  $C_3\text{-}C_6$  alkyl or  $C_3\text{-}C_6$  cycloalkyl, and  $x$  represents an integer from 1 to 5.

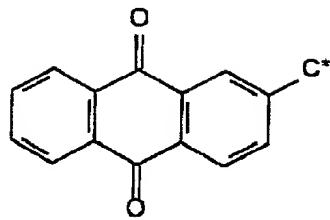
30 11. The reagent according to Claim 9, comprising at least one chromophore, corresponding to the general formula (II)



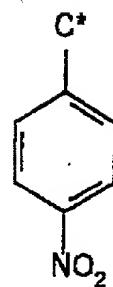
(II)



(III)



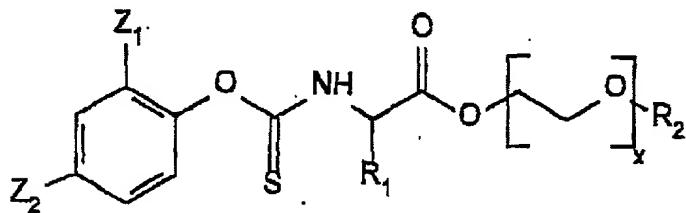
(IV)



(V)

in which  $Z_1$  and/or  $Z_2$  =  $NO_2$ ,  $R_1$  = phenyl,  $\alpha$ - or  
5  $\beta$ -indolyl, 1-naphthyl or 2-naphthyl and  $Y$  corresponds  
to any one of the formulae (III to V), the carbon by  
which  $Y$  is bonded to the oxygen of the carboxyl group  
of the enantiopure amino acid being marked by \*.

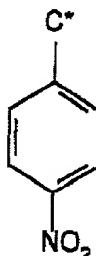
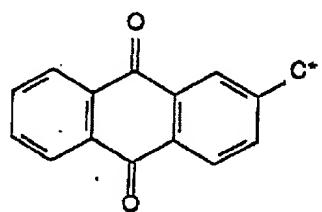
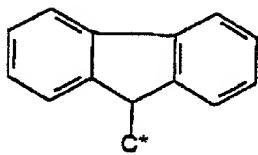
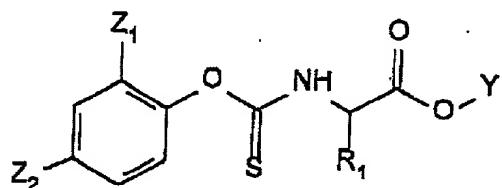
12. The reagent according to Claim 8 corresponding to  
10 the general formula (VI)



(VI)

in which  $Z_1$  and/or  $Z_2$  =  $\text{NO}_2$  or  $\text{F}$ ,  $R_1$  = phenyl,  $\alpha$ - or  $\beta$ -indolyl, 1-naphthyl or 2-naphthyl,  $R_2$  = Me, Et,  $C_3\text{-}C_6$  alkyl or  $C_3\text{-}C_6$  cycloalkyl and  $x$  represents an integer from 1 to 5.

- 5 13. The reagent according to Claim 8 comprising at least one chromophore, corresponding to the general formula (VII)



10

- in which  $Z_1$  and/or  $Z_2$  =  $\text{NO}_2$ ,  $R_1$  = phenyl,  $\alpha$ - or  $\beta$ -indolyl, 1-naphthyl or 2-naphthyl and  $Y$  corresponds to any one of the formulae (III to V), the carbon by which  $Y$  is bonded to the oxygen of the carboxyl group of the enantiopure amino acid being marked by \*.

14. A solution of the reagent according to Claim 8 in a polar organic solvent.

15. A method for the derivatization and separation of enantiomers of organic compounds comprising at least one free functional group wherein the solution

according to Claim 14 is used for the derivatization in an automatic device.

16. A process for the production of an enantiopure compound comprising at least one free functional group  
5 in which:

- (a) a mixture comprising the enantiomers of the compound comprising at least one free functional group is subjected to the separation process according to the invention
- 10 (b) a cleavage operation is carried out on a pure diastereomer obtained by separation of the mixture of diastereomers
- (c) the enantiopure compound is recovered.

ABSTRACT

Process for the separation of enantiomers and  
enantiopure reagent

Process for the separation of enantiomers comprising at least one free functional group, in which process a reagent based on an enantiopure amino acid is reacted in basic medium with a mixture comprising enantiomers.

(No figure)

**DECLARATION AND POWER OF ATTORNEY**

As a below named inventor, I declare that:

My residence, post office address, and citizenship are as stated below next to my name. I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter that is claimed and for which a patent is sought on the invention entitled:

"Process for the separation of enantiomers and enantiopure reagent"

[ ] the inventor's declaration for said application being executed concurrently with the execution of this instrument; said application to be filed in the U.S. Patent and Trademark Office;

[ ] said application having been filed in the U.S. Patent and Trademark Office on \_\_\_\_\_  
and given Application No. \_\_\_\_\_;

[ ] said application having been filed under the Patent Cooperation Treaty on \_\_\_\_\_  
and given Application No. \_\_\_\_\_, the United States of America having been designated.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge that duty to disclose information of which I am aware and which is material to the examination of the patent application in accordance with 37 CFR §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT International application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the space, any foreign application for patent or inventor's certificate, or of any PCT International application having a filing date before that of the application on which priority is claimed.

**Prior Foreign Application(s)**

Number	Country	Day/Month/Year Filed	Priority Claimed
<u>09900280</u>	<u>BELGIUM</u>	<u>21 APRIL 1999</u>	<u>X</u>
_____	_____	_____	_____

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below.

Application Serial Number	Filing Date
_____	_____

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s), or §365(c) of any PCT International application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose information known to me which is material to the patentability as defined in 37 CFR §1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

Application Serial Number	Filing Date	Status (patented, pending, abandoned)
_____	_____	_____

Each undersigned applicant hereby appoints the following attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: George H. Spencer (Registration No. 18,038), Robert J. Frank (Registration No. 19,112), Norman N. Kunitz (Registration No. 20,586), Gabor J. Kelemen (Registration No. 21,016), John W. Schneller (Registration No. 26,031), Marina V. Schneller (Registration No. 26,032), Robert Kinberg (Registration No. 26,924), L. Allen Wood, Jr. (Registration No. 28,134), Ashley J. Wells (Registration No. 29,847), James R. Burdett (Registration No. 31,594), Michael A. Gollin (Registration No. 31,957), Catherine M. Voorhees (Registration No. 33,074), Gary L. Shaffer (Registration No. 34,502), Chellis Erika Neal (Registration No. 36,877), G. Abc Zachariah (Registration No. 38,366), Patricia R. Brown (Registration No. 39,012), Julie A. Petruzzelli (Registration No. 40,769), Catherine A. Ferguson (Registration No. 40,877), Michael P. Leary (Registration No. 41,144), Michael A. Sartori (Registration No. 41,289), Zayd Alathari (Registration No. 42,256), Fei-Fei Chao (Registration No. 43,538) and Robert Babayi (Registration No. 33,471).

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The undersigned hereby authorizes the U.S. attorneys named herein to accept and follow instructions from the undersigned's assignee, if any, and/or, if the undersigned is not a resident of the United States, the undersigned's domestic attorney, patent attorney or patent agent, as to any action to be taken in the Patent and Trademark Office regarding this application without direct communication between the U.S. attorneys and the undersigned. In the event of a change in the persons(s) from whom instructions may be taken, the U.S. attorneys named herein will be so notified by the undersigned.

I declare (or certify, verify, or state) under penalty of perjury under the laws of the United States of America that the foregoing is true and correct.

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Inventor's signature \_\_\_\_\_ Date \_\_\_\_\_

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